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ARTICLE

Cloning and expression of kinesins from the thermophilic fungus *Thermomyces lanuginosus*

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Abbreviations: ATP, adenosine 5'-triphosphate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; , GTA; , thyleneglycol-bis-(beta-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; FPLC, fast protein liquid chromatography; 1PTG, isoprophyl-beta-d-thiogalactopyranoside; PCR, polymerase chain reaction; PMSF, phenylmethyl sulfonyl fluoride; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Abstract

The motor domain regions of three novel members of the kinesin superfamily TLKIF1, TLKIFC, and TLBIMC were identified in a thermophilic fungus *Thermomyces lanuginosus*. Based on sequence similarity, they were classified as members of the known kinesin families Unc104/KIF1, KAR3, and BIMC. TLKIF1 was subsequently expressed in *Escherichia coli*. The expression level was high, and the protein was mostly soluble, easy to purify, and enzymatically active. TLKIF1 is a monomeric kinesin motor, which in a gliding motility assay displays a robust plus-directed microtubule movement up to 2 μm/s. The discovery of TLKIF1 also demonstrates that a family of kinesin motors not previously found in fungi may in fact be used in this group of organisms.

Keywords: intracellular motility; kinesin; microtubules; motor protein; thermomyces

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Introduction

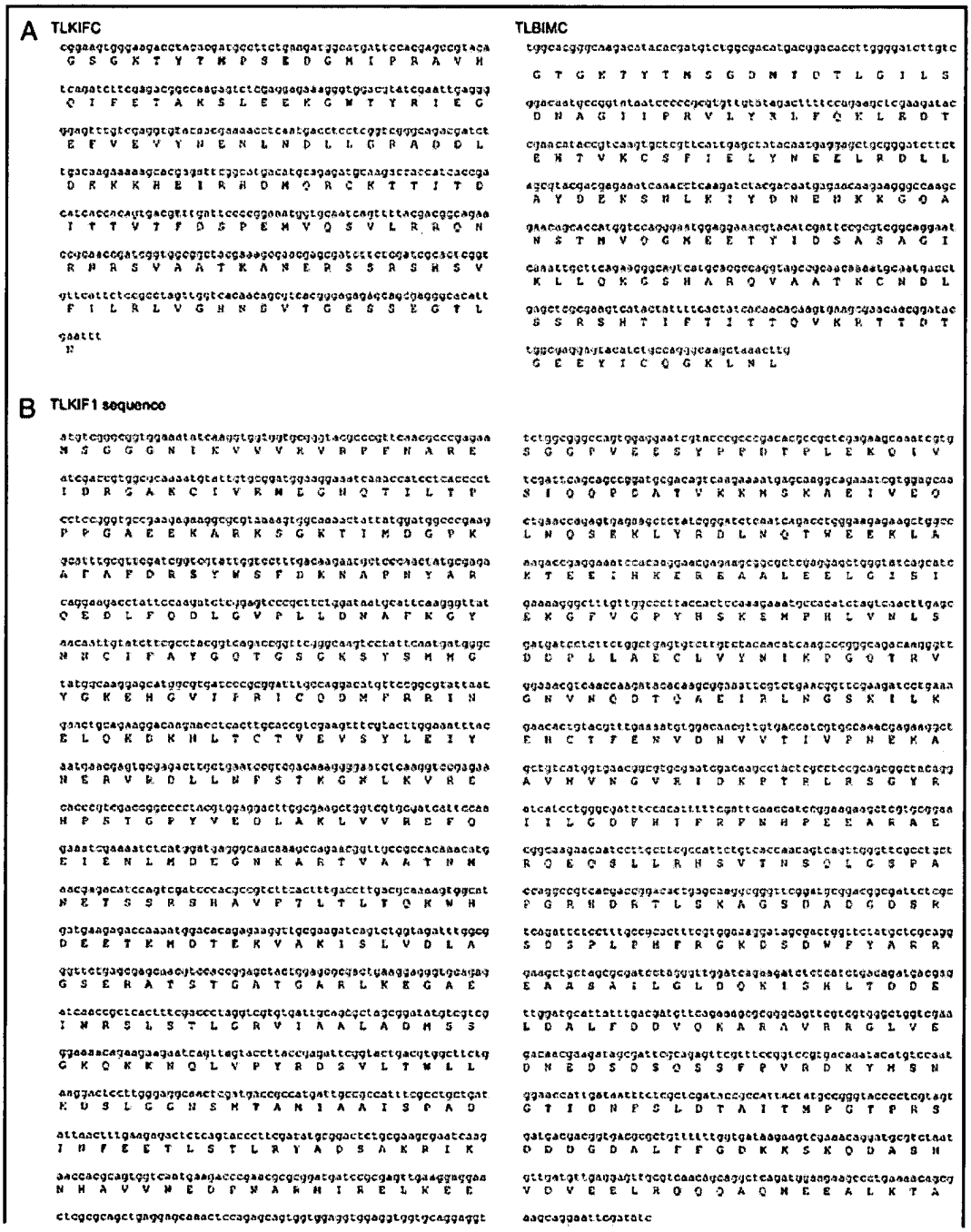
Kinesins constitute a diverse superfamily of motor proteins essential for many cellular functions including organization and maintenance of mitotic and meiotic spindles and transport of vesicles and organelles (Barton & Goldstein, 1996; Hirokawa, 1998; Goldstein & Philp, 1999). Intense effort to characterize the cellular functions of kinesins has been complemented by substantial progress in deciphering the mechanism of movement (Vale & Fletterick, 1997). A common feature of all members of the kinesin superfamily is the presence of a mechanochemical motor domain (Vale & Fletterick, 1997), which is necessary for binding to microtubules, movement, and force generation fueled by hydrolysis of ATP. The atomic structure of the kinesin motor domain was recently determined by X-ray crystallography (Kull et al., 1996; Sablin et al., 1996), opening the way to mechanistic analysis of motility. Further progress in the structural and kinetic studies of this group of proteins is made difficult by the somewhat fragile nature of kinesin enzymes. Many kinesins have been difficult, if not impossible to express in active form in bacteria, which severely limits the amount of pure protein available for studies.

For many other classes of proteins, stability problems were overcome by identifying, cloning, and expressing counterparts from a thermophilic organism (Kiefer et al., 1998). Enzymes from thermophiles are often more stable, express better in bacterial systems, and serve as a robust scaffold for mutagenesis studies. Because this approach was unexplored for kinesins, we isolated representative kinesins from a thermophilic organism. Even though members of the kinesin superfamily are restricted to the eukaryotic kingdom, which inhabits ecological niches much more tame than Archea and Procaryota, there are, nevertheless, eukaryotes whose enzymatic machinery operates at substantially elevated temperatures. One example is the thermophilic fungus *Thermomyces lanuginosus,* which tolerates temperatures up to 60 °C,

has a growth optimum of 50 °C, and will not grow below 30 °C (Deacon, 1997). *T. lanuginosus* was previously explored as a source of thermostable lipases (Berg et al., 1998), xylanases (Schlacher et al., 1996), and glucoamylases (Basaveswara Rao et al., 1981). It was also used as a source of ribosomal subunits for electron microscopic studies (Harauz & Flannigan, 1990). The demonstrated ability of *T. lanuginosus* to encode thermostable enzymes, and its relatively extreme thermophilicity (for a eukaryote) made *T. lanuginosus* an ideal candidate for a possible source of thermostable kinesins.

Results and discussion

Our initial polymerase chain reaction (PCR)-based screen discovered three novel kinesin-like proteins in *T. lanuginosus*, which we named TLKIF1, TLKIFC, and TLBIMC. Based on sequence homology in recovered regions of the motor domain (Fig. 1), these predicted proteins were most similar to motors from three different classes of kinesins (Table 1).



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